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Haematological Effects of Dichloromethane-Methanolic Leaf Extracts of *Carissa edulis* (Forssk.) Vahl in Normal Rat Models

Obel H Jorum[1,*], **Ngugi M Piero**[1] **and Alex K Machocho**[2]

[1]*Department of Biochemistry and Biotechnology, Kenyatta University, P.O Box 43844-00100, Nairobi, Kenya*

[2]*Department of Chemistry, Kenyatta University, P.O Box 43844-00100, Nairobi, Kenya*

[*]**Corresponding author:** Obel H. Jorum, Department of Biochemistry and Biotechnology, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya, Tel: +254 728 671 219; E-mail: humphreyobel@gmail.com

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Abstract

Assessment of hematological parameters can be used to explain blood related functions of a plant extract. The blood can act as pathological reflector and also as an indicator of the physiological state of an animal. Hematological disorders are increasingly on the rise while conventional management of these disorders is not easily accessible. This has prompted an increased use of medicinal plants which are readily available in the management of blood disorders. *Carissa edulis* (Forssk.) Vahl (Apocynaceae) like other terrestrial plants, has ethnopharmacological relevance and has been exploited by the local people in search for remedies for various diseases including those of the blood. Although *C. edulis* is widely used in managing blood related disorders in traditional system of medicine, no scientific research have been undertaken to evaluate its effects on the hematological profiles. This study therefore was designed to investigate the effects of Dichloromethane-Methanolic leaf extract of *C. edulis* on hematological parameters. Experimental rats were divided into four groups each consisting of five normal rats. The groups received oral doses of 50, 75 and 100 mg/kgbw of the extract while one group was used as control and did not receive any dosage. Hematological analysis and phytochemical screening were conducted using standard recommended procedures. The results of this study showed that DCM-MeOH leaf extract of *C. edulis* induced general increase in the levels of red blood cells, Hemoglobin and related parameter profiles across the different dose levels ($p<0.05$). The total and differential white blood cell counts also increased significantly at all the dose levels during the study period ($p<0.05$). Platelets and the related parameter levels significantly increased as well at all dose levels during this study period ($p<0.05$). Qualitative phytochemical screening confirmed the presence of various phytochemicals which included alkaloid, flavonoids, tannins, phenols, terpenes and traces of steroids which have the ability to protect the erythrocytes from oxidative damage as well as possess erythropoietin stimulatory, immune-stimulatory and thrombopoietic stimulatory activities. It was therefore concluded that the plant extract may be useful in the management of hematological disorders.

Keywords: *Carissa edulis*; Hematological parameters; Hematological disorders; Phytochemicals

Introduction

Haematology refers to the study of the number and morphology of the cellular elements of blood particularly; the red blood cells (erythrocytes), white blood cells (leucocytes) and the platelets (thrombocytes) in addition to the use of these results in the diagnosis and monitoring of diseases [1]. Haematological studies are of ecological and physiological interest in helping to understand the relationship of blood characteristics to the environment [2].

Erythrocytes have three main functions; to distribute oxygen to the periphery from the lungs through the pulmonary capillaries, remove carbon dioxide from the tissues back to the lungs through the systemic capillaries and to ensure that the acidic and basic values of the body are normal [3]. In order for the metabolic needs of tissues to be met, the appropriate amount of nutrients and oxygen must be available and supplied to the tissues [4].

White blood cells are the cells of the immune system. The name white blood cell derives from the physical appearance of a blood sample after centrifugation. All leukocytes are produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. White blood cells are best classified into two major lineages: the myeloid leukocytes and the lymphocytes [5].

Platelets are a component of blood whose main function is to stop bleeding by clumping and clogging blood vessel injuries [6]. They are 2-3 μm in greatest diameter [7].

Hematological complications consist mainly of abnormalities in the function, morphology and metabolism of erythrocytes, leukocytes and platelets [8]. Blood disorders that affect red blood cells include: Anemia which is characterized by low number of red blood cells and symptoms include fatigue, pale skin, and shortness of breath with exertion. Examples of anemia include, Iron deficiency anemia, anemia of chronic diseases, pernicious anemia (B12 deficiency), aplastic anemia, autoimmune hemolytic anemia, thalassemia, sickle cell anemia and polycythemia vera [9].

A range of disorders can cause decreases in white blood cells. This type of white blood cell decreased is usually the neutrophil. In this case the decrease may be called neutropenia or granulocytopenia. Neutropenia can be acquired or intrinsic and therefore a decrease in levels of neutrophils on lab tests is due to either decreased production of neutrophils or increased removal from the blood [10]. Lymphocytopenia is defined as total lymphocyte count below 1.0×10^9 g/l, the cells most commonly affected are CD4+ T cells. Like

neutropenia, lymphocytopenia may be acquired or intrinsic and there are many causes [11].

Most platelet disorders are due to an insufficient number of platelets, a condition known as thrombocytopenia. The classification of these disorders can be divided into two, congenital and acquired disorders [12]. Von Willebrand disease (VWD) is an autosomal dominant disorder and is the most common congenital bleeding disorder. Acquired disorders include myeloproliferative disorders. Platelet dysfunctions are observed in patients with chronic myeloproliferative disorders (essential thrombocythemia, polycythemia vera), but also in some patients with acute leukemia and myelodysplastic syndromes [12].

Most conventional ways of managing anemia, neutropenia and thrombocytopenia may be costly, have undesired side effects, painful to the patients or are not easily accessible. Blood transfusion limits its usefulness because of risk of infection, formation of antibodies which could complicate a later transplant or causes hemoglobin fluctuation [13]. Iron supplement used in anemic conditions often lead to diarrhoea, epigastric abdominal discomfort and in some cases, increase in infectious diseases morbidity in areas where bacterial infections are common [14]. Epogen used to treat anemia resulting from chronic kidney disease can lead to high blood pressure, crippling cluster mingraine, joint pain and clotting at the infection sites. Skin rash, flu-like symptoms, allergic reactions, seizures, thrombotic events are among other possible side effects [15].

In the view of all these setbacks, there is therefore need to develop agents that are effective, cheaply available and with negligible side effects as the alternative medical intervention. Unlike conventional drugs which are based on a single active ingredient targeting just one haematological component, the plant derived agents comprise of a cocktail of chemical compounds that act together to restore a normal physiological state. There is therefore urgent need to develop agents that are effective, cheaply available and with minimal side effects as alternative means of treatment.

Like other terrestrial plants, *Carissa edulis* has ethnopharmacological relevance and has also been exploited by the local people in the search for remedies for various ailments to increase vitality and manage hematological disorders [16]. Its effect in these activities has not been scientifically studied or validated. It was against this background that this study was undertaken to scientifically test the hematological claimed effect of the plant extracts in normal rats.

Materials and Methods

Collection and preparation of plant material

Fresh leaves of *Carissa edulis* were collected from Siakago division, Mbeere North Sub-County, Embu County, Kenya. The fresh leaves were identified with the help of local herbalists. The information gathered included vernacular names, plant parts used and the ailment treated. The samples were collected with acceptable bio-conservative methods and were properly sorted out, cleaned, and transported in polythene bags to Kenyatta University, Biochemistry and Biotechnology laboratories for drying and crushing. The plant samples were provided to an acknowledged Taxonomist for botanical authentication and a voucher specimen deposited at the Kenyatta University Herbarium, Nairobi, Kenya for future reference. The leaves of *C. edulis* were chopped into small pieces and air dried at room temperature for two weeks until properly dried. They were then ground into fine homogenous powder using an electric mill followed by sieving through mesh sieve and stored at room temperature awaiting extraction.

Extraction

For each sample, 200 g of powder was soaked separately in a cold 1:1 mixture of DCM and MeOH and stirred for six hours to extract the active compounds. The successive extract was filtered using whatman's filter papers and the filtrate concentrated under reduced pressure and vaccum using rotary evaporator. The concentrate was put in an airtight container and stored at -40°C before use in bioassay studies.

Experimental animals

Male healthy winstar rats (20), aged between two to three months and weighing an average of 150 g were used in this study. They were bred in the animal house of the Department of Biochemistry and Biotechnology, Kenyatta University. The rats were housed in cages, maintained under standard laboratory conditions of 12 hour light and dark sequence, at ambient temperature of 25 ± 2°C and 35-60% humidity. The animals were fed with standard rat pellets obtained from Unga Feeds Limited, Kenya, and water ad libitum. Ethical guidelines and procedures for handling experimental animals were followed.

Experimental design

The rats were randomly assigned into four groups where each group was having five normal rats. The groups were named A-D and were designed as follows: Group A was the control group and received normal saline (1 ml each) for 21 days. The other experimental groups were as follows; Group B animals were orally administered with the DCM-MeOH leaf extracts of *C. edulis* at a dose of 50 mg/kgbw for 21 days, Group C animals were orally administered with the DCM-MeOH leaf extracts of *C. edulis* at a dose of 75 mg/kgbw for 21 days and Group D animals were orally administered with the DCM-MeOH leaf extracts of *C. edulis* at a dose of 100 mg/kgbw for 21 days. The administration was done with the aid of a metal oropharyngeal cannula. Each rat was marked at the tail using a permanent marker pen to distinguish it from the lot. Daily cleaning of the cages was carried out.

Blood from rats in all groups was taken before the commencement of the first oral administration, then on the seventh, fourteenth and twenty-first days. During the entire period of study, rats were allowed free access to mice pellet and water ad libitum and observed for any signs of general illness, change in behaviour and/or mortality.

Preparation of extracts doses for administration

The dose level of 50 mg/kgbw was prepared by dissolving 0.038 g of the extract in 0.2 ml of 30% dimethylsulfoxide and topping up to 2.5 ml with distilled water, the dose level of 75 mg/kgbw was prepared by dissolving 0.056 g in 0.2 ml of 30% dimethylsulfoxide and topping up to 2.5 ml with distilled water while the dose level of 100 mg/kgbw was prepared by dissolving 0.076 g in 0.2 ml of 30% dimethylsulfoxide and topping up to 2.5 ml with distilled water.

Collection of blood samples

Blood samples were collected at the start of the experiment, then on the seventh day, the fourteenth day and finally on the twenty-first day from the tails of rats for the determination of hematological

parameters. The tails were first sterilized by swabbing with 70% ethanol and then the tip of the tails pierced. Bleeding was enhanced by gently milking the tail from the body towards the tip. Blood of approximately 2 ml was drawn into BD vacutainer® (BD Plymouth, UK) sample bottles containing anticoagulant (EDTA) for hematological parameters analysis. On the twenty-first day the animals were euthanized by use of chloroform.

Determination of hematological parameters

Hematological parameters and indices were determined from unclotted blood samples using standard protocols as described by [17]. Erythrocytes, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width and platelets, plateletcrit, mean platelet volume, platelet distribution width, white blood cell, neutrophils, monocytes, lymphocytes, eosinophils and basophils were determined using the Horiba ABX 80 Diagnostics (ABX pentra Montpellier, France).

Qualitative phytochemical screening

The extracts obtained were subjected to qualitative phytochemical screening to identify presence or absence of selected chemical constituents using methods of analysis as described by [18,19]. Standard screening tests for detecting the presence of different chemical constituents were employed. Secondary metabolites tested for were flavonoids, phenolics, saponins, alkaloids, cardiac glycosides, sterols and terpenoids.

Data management and analysis

Experimental data on different hematological parameters and serum lipid profiles were obtained from all the animals on the first day and compared with the seventh, fourteenth and twenty-first days for the three dose levels. It was recorded and tabulated on a broad spreadsheet sheet. Results were expressed as Mean ± Standard error of mean (SEM) for analysis. Statistical significance of difference among the groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to separate the means and obtain the specific significant differences among the different groups. The value of $P \leq 0.05$ was considered significant. Analysis of the data was done using Minitab statistical software, Version 17.

Results

Generally, during the twenty-one days of this experimental study, the levels of hematological parameters did not change significantly among the normal rat models in the control groups (Tables 1-6).

Effects of DCM-MeOH leaf extracts of *C. edulis* on erythrocytic and related parameter profiles in normal rats

Administration of DCM-MeOH leaf extracts of *C. edulis* induced significant changes in erythrocytes and related parameter profiles in normal rats (Table 1). Administration of the extract at the dose levels of 50, 75 and 100 mg/kgbw differently caused significant increase in the levels of RBC,HB, PCV, MCH, MCV, MCHC and RDW either after fourteen or twenty-one days ($p<0.05$; Table 1). These dose levels however did not cause any significant alteration in the level of erythrocytes and related parameters after seven days of extract administration (Table 1).

PARAMETERS	Control			50 mg/kgbw			75 mg/kgbw			100 mg/kgbw		
	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
RBC ($\times10^{12}$/l)	6.76 ± 0.12^{a}	6.43 ± 0.25^{a}	6.83 ± 0.05^{a}	6.71 ± 0.04^{a}	6.82 ± 0.02^{a}	6.83 ± 1.01^{a}	6.86 ± 0.076^{a}	7.13 ± 0.20^{ab}	7.05 ± 0.11^{ab}	6.88 ± 0.03^{a}	6.90 ± 0.03^{a}	6.80 ± 0.08^{a}
HB (g/dl)	15.76 ± 0.45^{a}	15.26 ± 0.15^{a}	15.34 ± 0.13^{a}	15.71 ± 0.18^{a}	15.65 ± 0.08^{a}	16.02 ± 0.08^{ab}	14.86 ± 0.09^{a}	15.16 ± 0.17^{a}	15.12 ± 0.14^{a}	15.12 ± 0.09^{a}	15.37 ± 0.18^{a}	15.48 ± 0.12^{a}
PCV (l/l)	49.00 ± 0.07^{a}	49.11 ± 0.07^{a}	49.56 ± 0.15^{a}	49.00 ± 0.12^{a}	49.31 ± 0.18^{a}	49.36 ± 0.18^{a}	49.06 ± 0.13^{a}	49.52 ± 0.16^{a}	49.56 ± 0.18^{a}	49.86 ± 0.10^{a}	50.34 ± 0.18^{ab}	50.14 ± 0.08^{ab}
MCV (fl)	71.24 ± 0.13^{a}	70.78 ± 0.22^{a}	71.65 ± 0.16^{a}	71.00 ± 0.19^{a}	72.00 ± 0.15^{b}	72.50 ± 0.16^{b}	71.94 ± 0.24^{a}	72.79 ± 0.18^{ab}	72.34 ± 0.26^{ab}	72.10 ± 0.08^{a}	72.38 ± 0.20^{a}	72.42 ± 0.14^{a}
MCH (pg)	22.00 ± 0.07^{a}	22.18 ± 0.12^{a}	22.04 ± 0.18^{a}	22.04 ± 0.09^{a}	22.41 ± 0.18^{a}	21.86 ± 0.85^{a}	22.88 ± 0.04^{a}	23.17 ± 0.18^{b}	22.66 ± 0.07^{a}	22.48 ± 0.16^{a}	22.66 ± 0.07^{a}	22.56 ± 0.10^{a}
MCHC (g/dl)	30.26 ± 0.17^{a}	30.14 ± 0.35^{a}	29.96 ± 2.05^{a}	31.34 ± 0.20^{a}	31.50 ± 0.07^{ab}	31.65 ± 0.17^{ab}	30.80 ± 0.04^{a}	30.99 ± 0.15^{a}	31.37 ± 0.18^{ab}	31.28 ± 0.18^{a}	31.56 ± 0.16^{a}	30.38 ± 0.18^{a}
RDW (%)	16.30 ± 0.27^{a}	16.26 ± 0.15^{a}	16.34 ± 0.09^{a}	16.76 ± 0.23^{a}	16.80 ± 0.04^{a}	16.68 ± 0.21^{a}	16.41 ± 0.22^{a}	16.82 ± 0.21^{ab}	16.59 ± 0.16^{a}	17.00 ± 0.11^{a}	17.25 ± 0.20^{ab}	17.32 ± 0.17^{ab}

Table 1: Effects of DCM-MeOH leaf extracts of *C. edulis* on erythrocytic and related parameter profiles in normal rats. All values are expressed as mean ± SEM for five animals per group. Values followed by the same superscript are not significantly different ($p>0.01$ analysed by ANOVA and Tukey's post hoc test). Means are compared among the days with the same extract dose level.

When the level of erythrocytes and related parameters were compared on specific day across all dose concentrations, it was evident that there were significant changes (Table 2). After seven, fourteen and twenty-one days of administration of the DCM-MeOH leaf extracts of

C. edulis, there was significant increase in the levels of all erythrocytes and related parameter profiles across all the dose levels ($p<0.05$; Table 2).

PARAMETERS	Day 7				Day 14				Day 21			
	Control	Dose 1	Dose 2	Dose 3	Control	Dose 1	Dose 2	Dose 3	Control	Dose 1	Dose 2	Dose 3
RBC ($\times10^{12}$/l)	6.76 ± 0.12^{a}	6.71 ± 0.04^{a}	6.86 ± 0.076^{a}	6.88 ± 0.03^{a}	6.43 ± 0.25^{a}	6.82 ± 0.02^{ab}	7.13 ± 0.20^{b}	6.90 ± 0.03^{ab}	6.83 ± 0.05^{a}	6.83 ± 1.01^{a}	7.05 ± 0.11^{ab}	6.80 ± 0.08^{a}
HB (g/dl)	15.76 ± 0.45^{a}	15.71 ± 0.18^{a}	15.86 ± 0.09^{a}	15.12 ± 0.09^{a}	15.26 ± 0.15^{a}	15.85 ± 0.08^{ab}	15.16 ± 0.17^{a}	15.37 ± 0.18^{a}	15.34 ± 0.13^{a}	16.02 ± 0.08^{b}	15.12 ± 0.14^{a}	15.48 ± 0.12^{a}
PCV (l/l)	49.00 ± 0.07^{a}	49.00 ± 0.12^{a}	49.06 ± 0.13^{a}	49.86 ± 0.10^{ab}	49.11 ± 0.07^{a}	49.31 ± 0.18^{a}	49.52 ± 0.16^{a}	50.34 ± 0.18^{b}	49.56 ± 0.15^{a}	49.36 ± 0.18^{a}	49.56 ± 0.18^{a}	50.14 ± 0.08^{ab}
MCV (fl)	71.24 ± 0.13^{a}	71.00 ± 0.19^{a}	71.94 ± 0.24^{ab}	72.10 ± 0.08^{ab}	70.78 ± 0.22^{a}	72.00 ± 0.15^{b}	72.79 ± 0.18^{b}	72.38 ± 0.20^{b}	71.65 ± 0.16^{a}	72.50 ± 0.16^{ab}	72.34 ± 0.26^{ab}	72.42 ± 0.14^{ab}
MCH (pg)	22.00 ± 0.07^{a}	22.04 ± 0.09^{a}	22.88 ± 0.04^{a}	22.48 ± 0.16^{a}	22.18 ± 0.12^{a}	22.41 ± 0.18^{ab}	23.17 ± 0.18^{b}	22.66 ± 0.07^{a}	22.04 ± 0.18^{a}	22.86 ± 0.85^{ab}	23.17 ± 0.18^{ab}	22.56 ± 0.10^{a}
MCHC (g/dl)	30.26 ± 0.17^{a}	31.34 ± 0.20^{ab}	30.80 ± 0.04^{ab}	31.28 ± 0.18^{ab}	30.14 ± 0.35^{a}	31.50 ± 0.07b	30.99 ± 0.15^{ab}	31.56 ± 0.16^{b}	29.96 ± 2.05^{a}	31.65 ± 0.17^{ab}	31.37 ± 0.18^{ab}	30.38 ± 0.18^{a}
RDW (%)	16.30 ± 0.27^{a}	17.06 ± 0.23^{ab}	16.61 ± 0.22^{ab}	17.08 ± 0.11^{ab}	16.26 ± 0.15^{a}	16.80 ± 0.04^{ab}	16.82 ± 0.21^{ab}	17.25 ± 0.20^{c}	16.54 ± 0.09^{a}	16.68 ± 0.21^{a}	16.59 ± 0.16^{a}	17.32 ± 0.17^{b}

Table 2: Effects of DCM-MeOH leaf extracts of *C. edulis* on erythrocytic and related parameter profiles in normal rats. All values are expressed as mean ± SEM for five animals per group. Values followed by the same superscript are not significantly different ($p>0.01$ analysed by ANOVA and Tukey's post hoc test). Means are compared among the extract dose levels with the same day. Dose 1: 50 mg/kgbw; Dose 2: 75 mg/kgbw; Dose 3: 100 mg/kgbw.

Effects of DCM-MeOH leaf extracts of *C. edulis* on total WBC and differential WBC count in normal rats

Administration of DCM-MeOH leaf extracts of *C. edulis* induced changes in total and differential WBC counts in normal rats (Table 3). Administration of the extract at the dose levels of 50, 75 and 100 mg/kgbw caused significant increase in the levels of WBC, lymphocytes, monocytes, neutrophils and eosinophils after fourteen and twenty-one days ($p<0.05$; Table 3). The levels of basophils, however, increased significantly after twenty-one days of extract administration at the dose levels of 75 and 100 mg/kgbw ($p<0.05$; Table 3).

PARAMETERS	Control			50 mg/kgbw			75 mg/kgbw			100 mg/kgbw		
	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
WBC ($\times10^{9}$/l)	7.66 ± 0.56^{a}	7.76 ± 0.50^{a}	8.28 ± 0.19^{a}	9.12 ± 0.43^{a}	12.14 ± 0.45^{b}	13.8 ± 0.57^{b}	9.22 ± 0.23^{a}	13.21 ± 0.23^{b}	15.24 ± 0.28^{c}	9.26 ± 0.30^{a}	12.2 ± 0.24^{b}	12.84 ± 0.52^{b}
Lymphocytes (%)	35.06 ± 0.27^{a}	35.44 ± 0.19^{a}	35.62 ± 0.24^{a}	37.4 ± 0.42^{a}	40.3 ± 0.34^{b}	41.3 ± 0.38^{b}	35.92 ± 0.95^{a}	39.88 ± 0.96^{b}	43.32 ± 0.79^{c}	36.59 ± 0.24^{a}	39.5 ± 0.28^{b}	41.45 ± 0.39^{c}
Monocytes (%)	27.54 ± 0.33^{a}	27.68 ± 0.19^{a}	27.72 ± 0.23^{a}	29.56 ± 0.55^{a}	32.94 ± 0.50^{b}	34.4 ± 0.65^{b}	29.06 ± 0.51^{a}	33.14 ± 0.51^{b}	36.76 ± 0.61^{c}	29.22 ± 0.12^{a}	32.28 ± 0.20^{b}	34.28 ± 0.46^{c}
Neutrophils (%)	3.86 ± 0.30^{a}	3.84 ± 0.29^{a}	3.87 ± 0.25^{a}	5.68 ± 0.32^{a}	8.7 ± 0.30^{b}	8.26 ± 0.20^{b}	6.18 ± 0.46^{a}	10.18 ± 0.48^{b}	8.3 ± 0.21^{ab}	5.88 ± 0.40^{a}	8.26 ± 0.20^{b}	8.19 ± 0.15^{b}
Eosinophils (%)	0.67 ± 0.02^{a}	0.65 ± 0.02^{a}	0.66 ± 0.03^{a}	0.79 ± 0.07^{a}	1.14 ± 0.08^{b}	1.39 ± 0.10^{b}	0.82 ± 0.03^{a}	1.17 ± 0.05^{ab}	1.51 ± 0.34^{ab}	0.81 ± 0.05^{a}	0.99 ± 0.08^{b}	1.23 ± 0.04^{ab}
Basophils (%)	0.13 ± 0.02^{a}	0.14 ± 0.02^{a}	0.12 ± 0.02^{a}	0.21 ± 0.02^{a}	0.24 ± 0.03^{a}	0.24 ± 0.02^{a}	0.20 ± 0.03^{a}	0.29 ± 0.02^{a}	0.61 ± 0.20^{b}	0.25 ± 0.03^{a}	0.29 ± 0.06^{a}	0.58 ± 0.33^{b}

Table 3: Effects of DCM-MeOH leaf extracts of *C. edulis* on total WBC and differential WBC counts in normal rats. All values are expressed as mean ± SEM for five animals per group. Values followed by the same superscript are not significantly different ($p>0.01$ analysed by ANOVA and Tukey's post hoc test). Means are compared among the days with the same extract dose level.

When the levels of total and differential WBC counts in normal rats were compared on specific day across all the dose concentrations, it was evident that there were significant changes (Table 4). After seven, fourteen and twenty one days of DCM-MeOH leaf extracts of *C. edulis* administration, there were significant increase in the levels of WBC, lymphocytes, monocytes, neutrophils, eosinophils and basophils at all the dose levels ($p<0.05$; Table 4). The levels of neutrophils, WBC, lymphocytes, monocytes and basophils, however, significantly decreased at dose levels of 100 mg/kgbw as compared to the dose level of 75 mg/kgbw after fourteen and twenty-one of extract administration ($p<0.05$; Table 4).

PARAMETERS	Day 7				Day 14				Day 21			
	Control	50 mg/ Kgbw	75 mg/ Kgbw	100 mg/ kgbw	Control	50 mg/ kgbw	75 mg/ kgbw	100 mg/ kgbw	Control	50 mg/ kgbw	75 mg/ kgbw	100 mg/ kgbw
WBC ($\times10^9$/l)	7.66 ± 0.56^a	9.12 ± 0.43^b	9.22 ± 0.23^b	9.26 ± 0.30^b	7.76 ± 0.50^a	12.14 ± 0.45^{ab}	13.21 ± 0.23^c	12.20 ± 0.24^b	8.28 ± 0.19^a	13.80 ± 0.57^b	15.24 ± 0.28^c	12.84 ± 0.52^{ab}
Lymphocytes (%)	35.06 ± 0.27^a	37.40 ± 0.42^b	35.92 ± 0.95^{ab}	36.58 ± 0.24^{ab}	35.44 ± 0.19^a	40.30 ± 0.34^b	39.88 ± 0.96^b	39.50 ± 0.28^b	35.62 ± 0.24^a	41.30 ± 0.38^b	43.32 ± 0.79^c	41.45 ± 0.39^b
Monocytes (%)	27.54 ± 0.33^a	29.56 ± 0.55^b	29.06 ± 0.51^b	29.22 ± 0.12^b	27.68 ± 0.19^a	32.94 ± 0.50^b	33.14 ± 0.51^b	32.28 ± 0.20^b	27.72 ± 0.23^a	34.40 ± 0.65^b	36.76 ± 0.61^c	34.28 ± 0.46^b
Neutrophils (%)	3.86 ± 0.30^a	5.68 ± 0.32^b	6.18 ± 0.46^b	5.88 ± 0.40^b	3.84 ± 0.29^a	8.70 ± 0.30^b	10.18 ± 0.48^c	8.26 ± 0.20^b	3.87 ± 0.25^a	8.26 ± 0.20^b	8.30 ± 0.21^b	8.19 ± 0.15^b
Eosinophils (%)	0.67 ± 0.02^a	0.79 ± 0.07^b	0.82 ± 0.03^b	0.81 ± 0.05^b	0.65 ± 0.02^a	1.14 ± 0.08^{ab}	1.17 ± 0.05^{ab}	0.99 ± 0.08^{ab}	0.66 ± 0.03^a	1.39 ± 0.10^{ab}	1.51 ± 0.34^{ab}	1.43 ± 0.04^{ab}
Basophils (%)	0.13 ± 0.02^a	0.21 ± 0.02^b	0.20 ± 0.03^b	0.25 ± 0.03^b	0.14 ± 0.02^a	0.24 ± 0.03^{ab}	0.29 ± 0.02^{ab}	0.29 ± 0.06^{ab}	0.12 ± 0.02^a	0.24 ± 0.02^{ab}	0.61 ± 0.20^b	0.58 ± 0.33^b

Table 4: Effects of DCM-MeOH leaf extracts of *C. edulis* on total WBC and differential WBC counts in normal rats. All values are expressed as mean ± SEM for five animals per group. Values followed by the same superscript are not significantly different ($p>0.01$ analysed by ANOVA and Tukey's post hoc test). Means are compared among the extract dose levels with the same day.

Effects of DCM- MeOH leaf extracts of *C. edulis* on platelets and related parameter profiles in normal rats

Administration of DCM-MeOH leaf extracts of *C. edulis* induced changes in platelets and related parameter profiles in normal rats (Table 5). Administration of extract at the dose levels of 50, 75, and 100 mg/kgbw caused significant increase in the levels of platelets, MPV, PCT, PDW after fourteen and twenty-one days ($p<0.05$; Table 5). The level of PCT, however, was not affected significantly by the dose level 100 mg/kgbw (Table 5). All the dose levels did not cause any change in the levels of platelets and related parameters after seven days of this study.

PARAMETERS	Control			50 mg/kgbw			75 mg/kgbw			100 mg/kgbw		
	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
PLATELETS ($\times10^9$/l)	794.60 ± 1.72^a	796.80 ± 1.36^a	796.40 ± 1.36^a	852.80 ± 2.08^a	872.80 ± 1.93^b	894.60 ± 2.04^c	860.20 ± 1.77^a	874.60 ± 1.63^b	896.20 ± 1.74^c	858.60 ± 3.09^a	875.20 ± 1.28^b	902.80 ± 1.93^c
PCT	0.26 ± 0.01^a	0.28 ± 0.01^a	0.28 ± 0.01^a	0.43 ± 0.04^a	0.59 ± 0.05^b	0.80 ± 0.05^c	0.63 ± 0.07^a	0.91 ± 0.08$^{a\ b}$	0.87 ± 0.07$^{a\ b}$	0.76 ± 0.05^a	0.89 ± 0.10^a	0.78 ± 0.07^a
MPV	4.72 ± 0.06^a	4.74 ± 0.09^a	4.75 ± 0.07^a	5.27 ± 0.20^a	6.51 ± 0.31^b	7.36 ± 0.29^c	6.92 ± 0.05^a	9.36 ± 0.20^b	9.08 ± 0.19^b	7.86 ± 0.16^a	9.24 ± 0.29^b	9.36 ± 0.20^b
PDW	17.90 ± 0.44^a	17.98 ± 0.6x2^a	17.92 ± 0.56^a	19.42 ± 0.23^a	21.60 ± 0.36^b	23.56 ± 0.49^c	21.54 ± 0.41^a	23.30 ± 0.24^b	25.38 ± 0.59^c	24.00 ± 0.35^a	26.10 ± 0.36^b	28.18 ± 0.24^c

Table 5: Effects of DCM-MeOH leaf extracts of *C. edulis* on platelets and related parameter profiles in normal rats. All values are expressed as mean ± SEM for five animals per group. Values followed by the same superscript are not significantly different ($p>0.01$ analysed by ANOVA and Tukey's post hoc test). Means are compared among the days with the same extract dose level.

When the level of platelets and related parameter profiles in normal rats were compared on specific day across all the dose concentrations, it was evident that there were significant changes (Table 6). After seven, fourteen and twenty one days of administration of the DCM-MeOH leaf extracts of *C. edulis*, the levels of platelets, PCT and PDW were significantly increased at all the dose levels ($p<0.05$; Table 6). The levels of MPV also increased significantly at the dose levels of 75 and 100 mg/kgbw ($p<0.05$; Table 6).

PARAMETERS	Day 7				Day 14				Day 21			
	Control	Dose 1	Dose 2	Dose 3	Control	Dose 1	Dose 2	Dose 3	Control	Dose 1	Dose 2	Dose 3
PLATELETS ($\times 10^9$/l)	794.6 ± 1.72^a	852.80 ± 2.08^b	860.20 ± 1.77^b	858.60 ± 3.09^b	796.8 ± 1.36^a	872.80 ± 1.93^b	874.60 ± 1.63^b	896.20 ± 1.74c	796.4 ± 1.36^a	894.60 ± 2.04^b	896.20 ± 1.74^b	902.80 ± 1.93^b
PCT (%)	0.26 ± 0.01^a	0.43 ± 0.04^b	0.63 ± 0.07^{b}c	0.76 ± 0.05^{b}c	0.28 ± 0.01^a	0.59 ± 0.05^{ab}	0.91 ± 0.08^b	0.89 ± 0.10^{ab}	0.28 ± 0.01^a	0.8 ± 0.05^b	0.87 ± 0.07^b	0.78 ± 0.07^b
MPV (fL)	4.72 ± 0.06^a	5.27 ± 0.19^a	6.92 ± 0.05^b	7.86 ± 0.16^b	4.74 ± 0.09^a	6.51 ± 0.31^b	9.36 ± 0.20c	9.24 ± 0.29c	4.75 ± 0.067^a	7.36 ± 0.28^b	9.08 ± 0.19^b	9.36 ± 0.20^b
PDW (%)	17.9 ± 0.44^a	19.42 ± 0.23^b	21.54 ± 0.41^b	24 ± 0.35c	17.98 ± 0.62^a	21.6 ± 0.36^b	23.30 ± 0.24^b	26.1 ± 0.36^{b}c	17.92 ± 0.56^a	23.56 ± 0.49^b	25.38 ± 0.59^b	28.18 ± 0.24c

Table 6: Effects of DCM-MeOH leaf extracts of *C. edulis* on platelets and related parameter profiles in normal rats. All values are expressed as mean ± SEM for five animals per group. Values followed by the same superscript are not significantly different (p>0.01 analysed by ANOVA and Tukey's post hoc test). Means are compared among the extract dose levels with the same day. Dose 1: 50 mg/kgbw; Dose 2: 75 mg/kgbw; Dose 3: 100 mg/kgbw.

Phytochemical screening

Qualitative phytochemical screening of the DCM-MeOH leaf extracts of *C. edulis* revealed the presence of alkaloids, flavonoids, phenolics, terpenoids and traces of steroids. However, saponins and cardiac glycosides were absent in the leaf extracts (Table 7).

Phytochemicals	Presence/Absence
Alkaloids	++
Flavonoids	++
Steroids	+ (trace)
Saponins	-
Cardiac glycosides	-
Phenolics	++
Terpenoids	++

Table 7: Phytochemical composition of DCM-MeOH leaf extract of *Carissa edulis*. Present phytochemicals are denoted by (++) sign, absent phytochemicals are denoted by (-) sign while + (trace) denote slightly present phytochemicals.

Discussion

Assessment of hematological parameters can be used to explain hematological functions of a chemical compound or plant extracts in an organism [20]. Blood act as a pathological reflector of the status of exposed animals to toxicants and other conditions and/or agents [21]. In the present study, the DCM-MeOH leaf extracts of *C. edulis* demonstrated varying degrees of hematological parameters changes in normal rats at the dose levels of 50, 75 and 100 mg/kgbw.

The extract of *C. edulis* significantly increased the levels of RBC, Hb, PCV, MCV, MCH, MCHC and RDW in normal rats. The observed increases in RBC, Hb and PCV levels upon administration of DCM-MeOH leaf extract of *C. edulis* suggests that the extract could have stimulated erythropoietin release in the kidney, which is the humoral regulators of RBC production [22]. Expectedly, increase in RBC counts on administration of *C. edulis* extract resulted in increase in MCV levels while increase in Hb levels resulted to increase in MCH and MCHC profiles. Since MCHC, MCH and MCV profiles relate to individual red blood cell count while hemoglobin and hematocrit profiles relate to the total population of red blood cells in the blood, it could thus imply that though the extract may stimulate the production of red blood cells and hemoglobin, it could have an inhibitory effect on hemoglobin incorporation into red blood cells and a consequent reduction in oxygen exchange [23].

The presences of phytochemicals like flavonoids, tannins and terpenes in the dichloromethane; methanolic leaf extracts of *C. edulis* may be responsible for the haemopoietic stimulating effects [24]. This is in line with previous research that showed that prophylactic and therapeutic oral administration of antioxidant supplements of plant extracts significantly increased cells of hematopoietic origin in animals exposed to potentially lethal dose of radiation [25]. Flavonoids, tannins and terpenes have been found to protect erythrocytes from oxidative damage [26]. Further [27,28] reported that flavonoids have various benefits for human health due to their antioxidant and free-radical scavenging activities as well as anti-inflammatory, antiviral and anti-cancer properties [29].

Administration of DCM-MeOH leaf extract of *C. edulis* led to significant increase in the levels of white blood cells and all related indices in normal rats. This shows that the DCM-MeOH extracts of *C. edulis* may have immune boosting properties. The increase in WBC count may have been due to enhancement in the rate of entry of leucocytes into the blood from the bone marrow and a diminished rate of removal from circulation. Granulocyte-macrophage colony stimulating factor, macrophage colony stimulating factor, interleukins (IL-2, IL-4 and IL-5) regulate the proliferation, differentiation and maturation of committed stem cells responsible for the production of WBCs [30]. Therefore, such increase in WBC counts may be due to overproduction of these haematopoietic regulatory elements by the stromal cells and macrophages in the bone marrow [31]. These stimulant effects could be associated with the adjuvant activity of some phytochemicals found in the extracts. Alkaloids, tannins, phenolic compounds and flavonoids have generally been reported as immunostimulants [32,33].

Differential white blood cell counts are indicators of the ability of an organism to eliminate infection. An increase in the number of circulating leukocytes is rarely due to an increase in all the types of leukocytes. Neutrophils attack and destroy pathogens in the blood [34]. The increased neutrophil counts improve the phagocytic activity in the animals. Lymphocytes are the main effector cells of the immune system. The increase in the lymphocyte levels in the present study may help improve the effector cells of the immune system. Similarly, increased levels of eosinophils and basophils observed in the present study may suggest positive effect on the immune system. Since monocytes have been shown to increase in cases of infection, the increase in monocytes observed with the extract in this study may be ascribed to challenges on the immune system.

Platelets are the blood cells involved in Coagulation [35]. Coagulation of blood requires that the platelets should be in sufficient size, number and function. The increase in the platelet levels observed in this study may be explained by stimulatory effect on thrombopoietin [36]. Bone marrow is responsible for the production of red blood cells, white blood cells and platelets [37]. The significant increase in platelets and related parameter profiles after oral administration of DCM-MeOH leaf extracts of *C. edulis* suggests that the extracts contain compounds and phytochemicals that may have stimulated thrombopoietic process in normal rats.

The significant increase in platelet count may indicate that the extracts have the potential to be developed as plant based therapeutic agents for thrombocytopenia. This is in line with [38], who reported that leaf juice of Carica papaya consumed during the course of dengue infection had the potential to induce platelet production. An increase in platelet count after seven days of oral administration DCM-MeOH leaf extracts of C. edulis extract may indicate that the extract had a megakarypoietic stimulatory activity. This is in line with finding of [39] that platelets are produced from megakaryocytes within 4 to 6 days under normal healthy body conditions.

Generally, the significant increase in platelets, plateletcrit and MPV profiles after oral administration of DCM-MeOH leaf extracts of *C. edulis* may be attributed to presence of tannins, which have been shown to confer antihemorrhagic properties in animals. This agrees with the findings of Vijaya [40], on extract of Euphorbia hirta used in promoting the development of blood platelets, stopping hemorrhage and preventing further bleeding.

Conclusions

In conclusion, the present study showed that oral administration of DCM-MeOH leaf extract of *C. edulis* in normal rats;

Resulted in a significant increase in the levels of erythrocytic parameter profiles. This may suggest that the plants possess erythropoietin stimulating activity and phytochemicals that also improve haematopoeitic activity of the cells and the improvement in erythrocyte membrane integrity through the antioxidant potential of the extract, thereby reducing haemolysis hence can play a vital role in management and or prevention of anemia.

That the significant increase in total white blood cell and differential white blood cell counts in normal mice after oral administration of the extract shows that the DCM-MeOH leaf extracts of *C. edulis* may promote the immune-stimulatory activities. These stimulant effects could be associated with the adjuvant activity of some phytochemicals found in the extracts hence can be used in management of immune-dependent disorders.

That the significant increase in platelet and related parameter profiles in normal mice after oral administration of the DCM-MeOH leaf extracts of *C. edulis* shows that the extract has the potential to stimulate thrombopoietin production and can thus be used to manage hemostatic capacity of blood.

The present study, therefore, scientifically confirms and supports the traditional use of leaves of *C. edulis* in enhancing hematological parameters and generally improving health.

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